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# TECHNETIUM-99m PYROPHOSPHATE: COMPARISON OF $ED_{50}$ FOR TETANY AND ACIDOSIS WITH ACUTE $LD_{50}$

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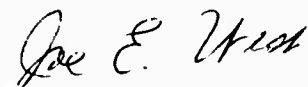
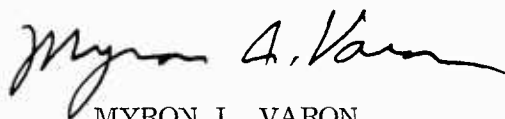# TECHNETIUM-99m PYROPHOSPHATE: COMPARISON OF $ED_{50}$

# FOR TETANY AND ACIDOSIS WITH ACUTE $LD_{50}$

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## ACKNOWLEDGMENT

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# FOREWORD
## (Nontechnical summary)

Tetany and metabolic acidosis occur acutely after toxic doses of technetium-99m Sn-pyrophosphate. To quantify the dose effect relations of these phenomena, measurements of serum calcium and $PCO_2$ were made following the administration of technetium-99m Sn-pyrophosphate intravenously, as a single bolus, on a milligram per kilogram body weight basis.

The $LD_{50/5\ minutes}$ for pyrophosphate was found to be 41.0 mg/kg with 95 percent confidence limits of 39.4 to 42.7 mg/kg body weight. The $LD_{50/5\ minutes}$ for polyphosphate was also determined and was found to be 29.4 mg/kg with 95 percent confidence limits of 27.4 to 31.5 mg/kg body weight. Plots evaluating the slopes of the two compounds showed no significant statistical difference. Polyphosphate was 1.4 times as toxic as the pyrophosphate agent on an $LD_{50/5\ minutes}$ basis.

Acidity measurements revealed that the plasma serum $PCO_2$ remained between 30 and 40 torr until doses of pyrophosphate exceeded 35 mg/kg body weight, after which it fell sharply. Serum calcium levels were measured by atomic absorption spectroscopy and electrocardiography. Both techniques revealed a significant drop in the values of ionized and bound calcium at a dosage of 20 mg/kg or greater. The clinical symptomatology consistent with tetany did not occur, however, until the dosage of 22 mg/kg body weight of the pyrophosphate compound was reached. It is therefore concluded that determination of toxic effects of phosphate agents should not be based on the $LD_{50}$ alone, but on dosages which produce hypocalcemia and metabolic acidosis.

ABSTRACT

Following the administration of toxic doses of technetium-99m Sn-pyrophosphate in rats, clinical manifestations are consistent with tetany and metabolic acidosis. To evaluate these phenomena, the acute $LD_{50/5 \text{ minutes}}$ was determined and serum calcium and arterial plasma $PCO_2$ were recorded and compared to the acute $LD_{50/5 \text{ minutes}}$. The $LD_{50/5 \text{ minutes}}$ for polyphosphate was found to be $29.4 \pm 2.0$ mg/kg body weight. The $LD_{50/5 \text{ minutes}}$ for pyrophosphate was found to be $41.0 \pm 1.6$ mg/kg body weight (2 S.D.). There was no significant difference between the slopes of the two compounds. When acute $LD_{50}$'s were compared, polyphosphate was 1.4 times as toxic as pyrophosphate. Arterial blood $PCO_2$ remained between 30 and 40 torr until dosages of pyrophosphate exceeded 35 mg/kg body weight, after which it fell sharply. Serum calcium was monitored by atomic absorption spectroscopy and electrocardiography. There was a significant drop in the ionized and bound calcium beginning at concentrations of an administered dose of 20 mg/kg body weight. However, tetany was not apparent until 22 mg/kg body weight of pyrophosphate were given. Therefore, evaluation of the toxic effects of phosphate agents should not be limited to determination of $LD_{50}$ alone, but should include appropriate biochemical measurements in blood.

# I. INTRODUCTION

$^{99m}$Tc phosphate agents are now considered to be the radiopharmaceuticals of choice for bone scanning. In a recent report on $^{99m}$Tc labeled pyrophosphate for skeletal imaging, it was shown that by systematically varying the parameters of $^{99m}$Tc Sn-pyrophosphate preparations the best method of preparation was obtained by adding 1.75 ml of 0.1 M sodium pyrophosphate (pH 10) to 0.25 ml of 1 mg/ml stannous chloride followed by 1.50 ml $^{99m}$TcO$_4^-$.[4] In vivo organ distribution analysis in rabbits indicated the importance of using doses on a milligram per kilogram basis for comparative animal studies. The authors concluded that if a proper dose, on a milligram per kilogram basis, of $^{99m}$Tc Sn-pyrophosphate is used high quality bone images in both animals and humans can be obtained.

This report deals with the evaluation of acute tetany and metabolic acidosis which were found in Sprague-Dawley rats after toxic doses of $^{99m}$Tc Sn-pyrophosphate were administered intravenously as a single bolus on an increasing milligram per kilogram dosage up to and exceeding the LD$_{50}$ level.

# II. MATERIALS AND METHODS

The chemical nature and method of compounding $^{99m}$Tc Sn-pyrophosphate and $^{99m}$Tc Sn-polyphosphate radiopharmaceuticals used in this study have previously been summarized.[2,4] The pyrophosphate dose is given in milligrams of Na$_4$P$_2$O$_7$10H$_2$O (mw = 446) per kilogram body weight. Determination of the acute LD$_{50}$ for each agent was made using Sprague-Dawley rats with a body weight range of 171-246 grams. Each rat was mildly anesthetized with 25 mg sodium phenobarbital administered

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intraperitoneally. Each compound was administered intravenously as a single bolus on an increasing milligram per kilogram dose level by injections into the femoral vein following a surgical cutdown.

Total serum calcium levels were measured by atomic absorption spectroscopy[10] using the Jarrell-Ash Model 280 Atomsorb.* Total serum protein levels were determined by the biuret reaction adapted to the AutoAnalyzer† by Sobocinski et al.[12] Ionized serum calcium levels were evaluated by two methods. The first method incorporated serial electrocardiographic monitoring on the Sprague-Dawley rats prior to and following administration of the pyrophosphate compound. Each electrocardiogram was obtained from standard limb leads using cutaneously implanted electrodes and a two-channel direct electric writing rectilinear paper recorder from the Sanborn recording system. The second method involved calculation of the $Ca^{++}$ content from the relationship between calcium and protein using the following formula based on the original McLean and Hastings equation:[8]

$$mg\ Ca^{++}/100\ ml = \frac{6\ Ca - (P/3)}{P + 6}$$

where Ca equals milligrams of total Ca/100 ml, and P equals grams of protein/100 mg.

Metabolic acidosis was evaluated by measuring arterial $PCO_2$ levels. Blood samples were drawn from the abdominal aorta 5 minutes after intravenous injection of the pyrophosphate compound. Each sample was analyzed using the blood microsystem in conjunction with the BMS 3 MK 2 Acid/Base Analyzer (Radiometer-Copenhagen).

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\* Jarrell-Ash Division, Fisher Scientific Company, Waltham, Massachusetts
† Technicon Corporation, Tarrytown, New York

Control animals received an equal volume of the vehicle and anesthesia. The effect of anesthesia on the blood constituents was evaluated separately.

The experimental results were evaluated by the Student's "t" test with the Behrens-Fisher modification.[1] If the chance occurrence was 5 percent or less, the data were considered significant.

## III. RESULTS

$LD_{50}$ study. Bioassays were carried out on Sprague-Dawley rats to examine the toxicity of polyphosphate and pyrophosphate. Five dose levels of polyphosphate were selected, and the results are shown in Table I. The $LD_{50/5 \text{ minutes}}$ was found to be 29.4 mg/kg with 95 percent confidence limits of 27.4 to 31.5 mg/kg body weight. The dose mortality curve and its confidence interval are plotted in Figure 1. Pyrophosphate was also administered at five dosage levels (Table I) and the $LD_{50/5 \text{ minutes}}$ was found to be 41.0 mg/kg with 95 percent confidence limits of 39.4 to 42.7 mg/kg body weight. The dose response curve is also presented in Figure 1 for comparison. The $LD_{50}$ calculations were performed using the probit analysis method described by Finney.[5] The slopes of the two curves were compared and found not to differ significantly. Polyphosphate was 1.4 times as toxic as the pyrophosphate over the dosage range examined.

Acidity measurements. Measurements of serum $PCO_2$ and pH were made 5 minutes after intravenous injections of pyrophosphate using the Acid/Base Analyzer. Figure 2 shows that the $PCO_2$ levels for the most part remain between 30 and 40 torr until dosages exceeding 35 mg/kg of body weight after which they fell sharply. The pH (Figure 3) remained between 7.3 and 7.5 but increased sharply with pyrophosphate

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levels in excess of 35 mg/kg body weight. These observations are consistent with the $LD_{50}$ value of 44 mg/kg body weight since later changes in serum acidity are associated with rapid death.

Hypocalcemia measurements. Another factor contributing to the acute toxicity of pyrophosphate, previously observed by Gosselin et al.,[7] is reduction in the plasma level of ionized calcium by the binding of $Ca^{++}$ by pyrophosphate. This type of hypocalcemia is difficult to detect by chemical procedures so an additional biological index was added to study this phenomenon. The electrocardiogram is one of the more

Table I. Dose-Mortality Data

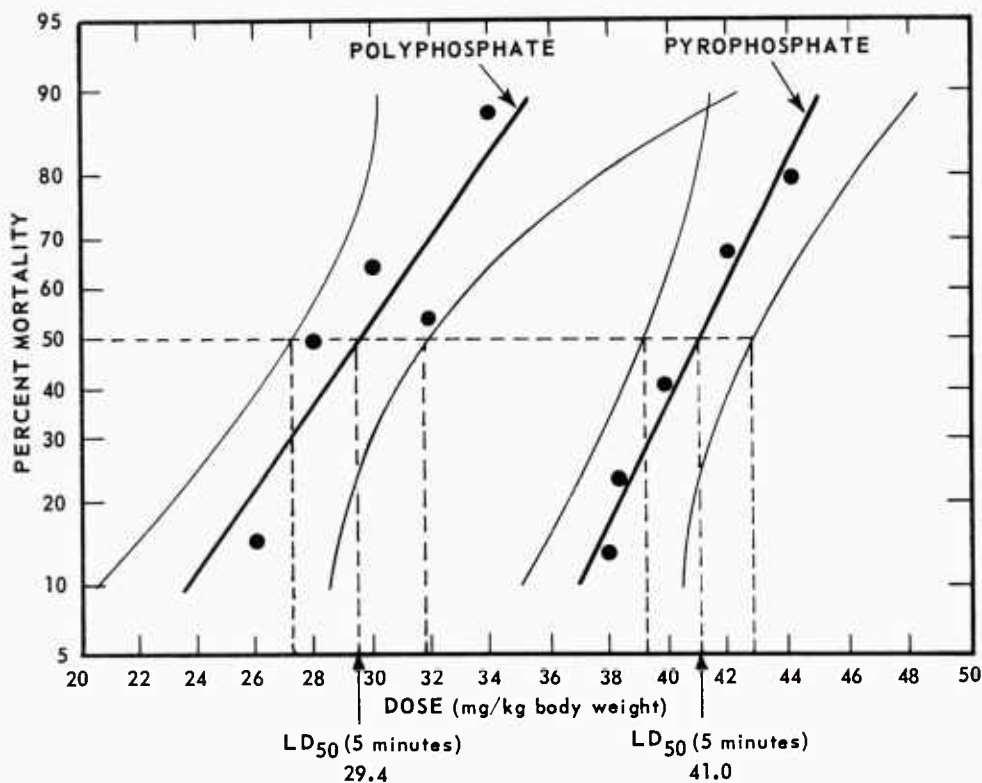| Dose mg/kg body weight | Number tested | Number dead | Percent mortality |
|---|---|---|---|
| Polyphosphate | | | |
| 26 | 14 | 2 | 14 |
| 28 | 12 | 6 | 50 |
| 30 | 11 | 7 | 64 |
| 32 | 11 | 6 | 55 |
| 34 | $\frac{9}{57}$ | 8 | 89 |
| $LD_{50/5 \text{ min}}$ = 29.4 mg/kg body weight | | | |
| Pyrophosphate | | | |
| 36 | 8 | 0 | 0 |
| 38 | 7 | 1 | 14 |
| 40 | 10 | 4 | 40 |
| 42 | 9 | 6 | 67 |
| 44 | $\frac{10}{44}$ | 8 | 80 |
| $LD_{50/5 \text{ min}}$ = 41.0 mg/kg body weight | | | |

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Figure 1. Plot of acute mortality (5 minutes) of Sprague-Dawley rats receiving intravenous injections of polyphosphate and of pyrophosphate. Time of death is within 5 minutes after injection. Each point represents the percent mortality of 7 to 14 animals.

sensitive devices for evaluating the presence of hypocalcemia. The electrocardiographic change consistent with the development of hypocalcemia is prolongation of the Q-T interval.[11] It is important to emphasize that the prolonged Q-T interval of hypocalcemia is a result of lengthening of electrical systole. It is mainly the prolongation of the S-T segment rather than widening of the T wave.[7] The T wave is generally normal in hypocalcemia. This often results in partial fusion of the T wave with the subsequent P wave. As seen in Figure 4, as the dose of pyrophosphate on a milligram

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per kilogram basis was increased, the Q-T interval lengthened. Other electrocardiogram abnormalities which were noted included preventricular contractions, tachycardia, ventricular fibrillation, and finally cardiac standstill. The electrocardiographic hypocalcemic effects were not present until the dosage was 12 mg/kg or greater. Tetany, however, did not manifest itself until the dosage was 22 mg/kg or greater.
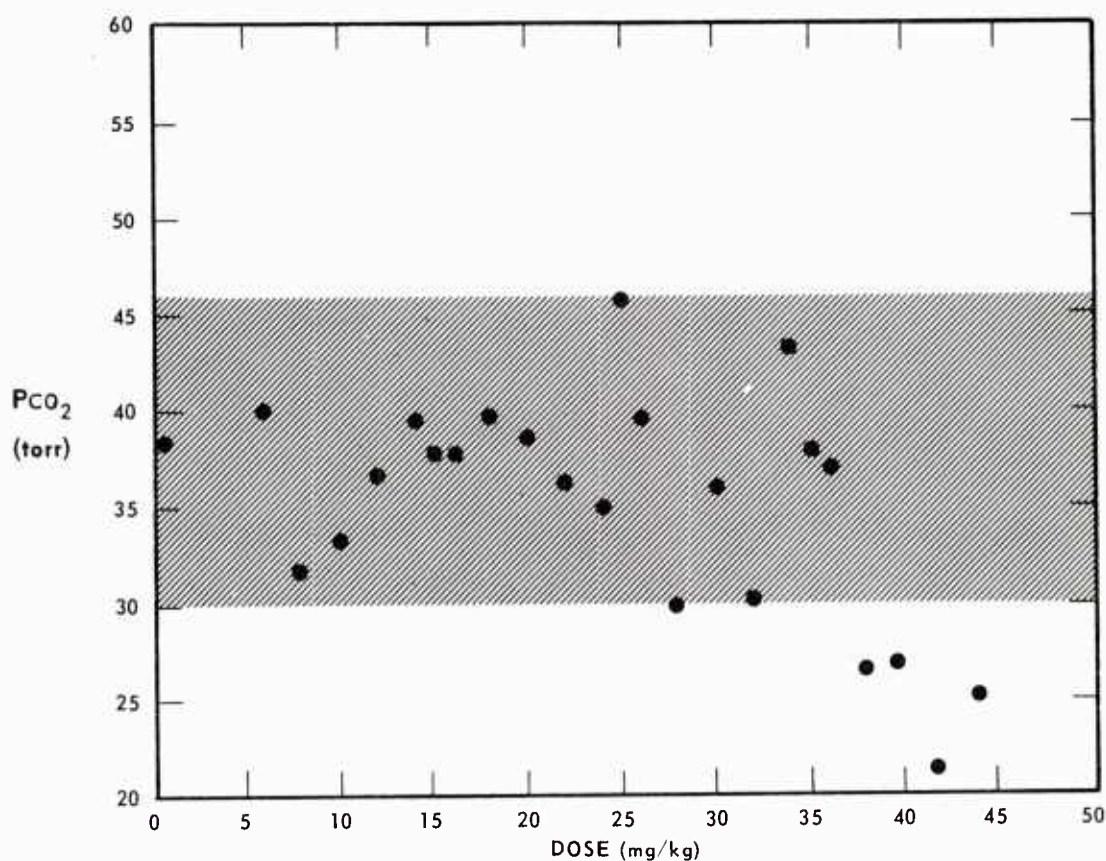


Figure 2. Arterial blood $PCO_2$ levels in rats 5 minutes following intravenous injection of pyrophosphate. Each point represents the mean of 2 to 10 animals. Hatched area represents normal range ($\overline{x} \pm 2$ S.D.) of control rats.
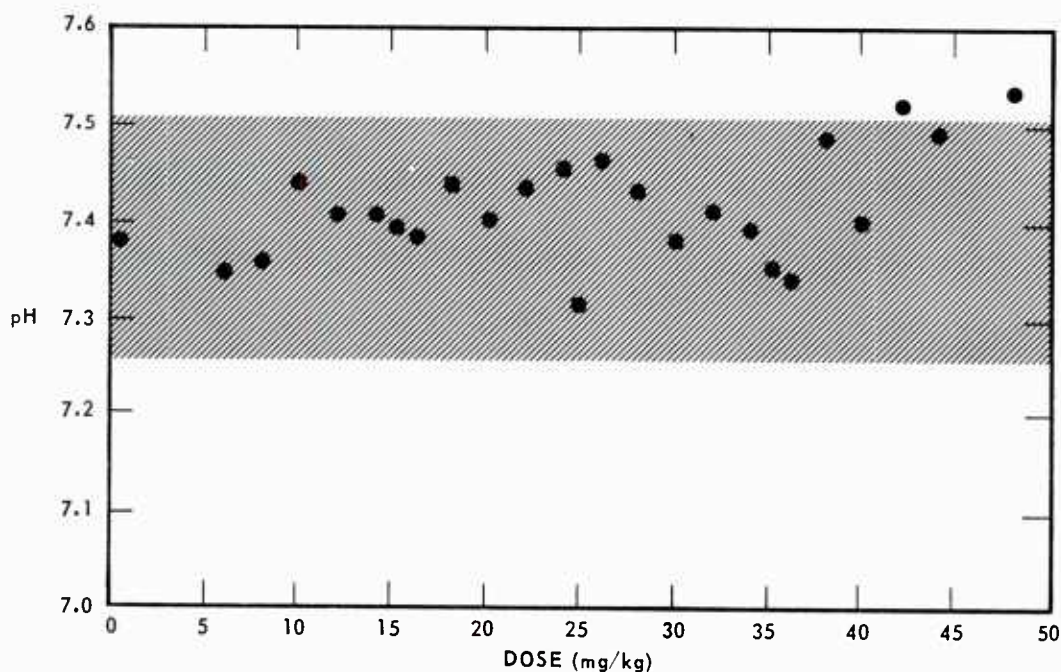
Figure 3. Arterial blood pH 5 minutes following intravenous
injection of pyrophosphate. Each point represents
the mean of 2 to 10 animals. Hatched area repre-
sents normal range ($\overline{x} \pm 2$ S.D.) of control rats.

Evaluation of the serum levels of total calcium, ionized calcium and protein
revealed a significant drop in the serum bound and ionized calcium levels, beginning
at a dosage of approximately 20 mg/kg (Figures 5 and 6). Both total and ionized cal-
cium levels followed a similar pattern with respect to dosages. The values for per-
cent ionized calcium throughout the dose ranges tested remained around 38 to 42 per-
cent which would be expected when both the total calcium and ionized calcium levels
decreased similarly (Figure 7). When $CaCl_2$ was administered intravenously, the
hypocalcemic effect could be reversed, and the lengthened Q-T interval could be re-
turned promptly to its normal duration (Figure 4). This could only be accomplished

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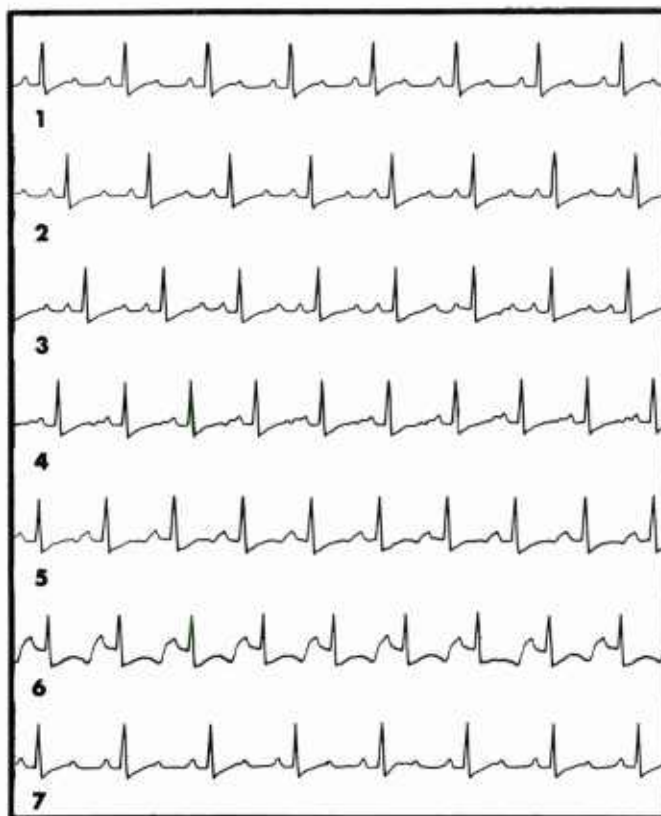
Figure 4. Representative electrocardiograms from male
Sprague-Dawley rats receiving 10 mg/kg (1),
14 mg/kg (2), 16 mg/kg (3), 18 mg/kg (4),
20 mg/kg (5), 41 mg/kg (6) (body weight) pyro-
phosphate intravenously as a single bolus. Fol-
lowing intravenous administration of $CaCl_2$ the
electrocardiogram (6) returned to a normal
pattern (7).

if the $CaCl_2$ was administered before the appearance of any severe cardiac arrhythmia.

Because the Q-T interval is also recognized to be a function of the cycle length[7] and

since the precise relationship between the two is unknown for the Sprague-Dawley rat,

the Q-T changes, shown in Figure 4, include only those while the heart rate remains

approximately 15 percent of the preinjection rate. The appearance of cardiac

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BOUND CALCIUM (mg/dl)

10

8

6

4

2

0

0   5   10   15   20   25   30   35   40   45   50

DOSE (mg/kg)

LD50=41mg/kg

Figure  5.  Serum-bound calcium concentrations 5 minutes following intravenous injection of pyrophosphate.  Each point represents the mean of 2 to 10 animals.  Hatched area represents normal range ($\overline{x}$ + 2 S.D.) of control rats.

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IONIZED CALCIUM (mg/dl)

10

8

6

4

2

0

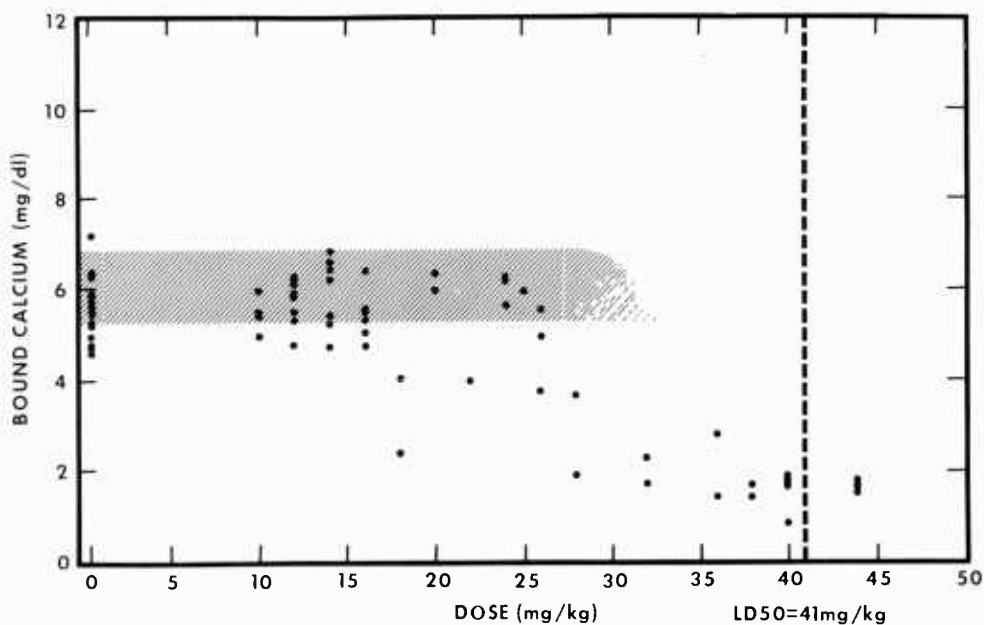0   5   10   15   20   25   30   35   40   45   50
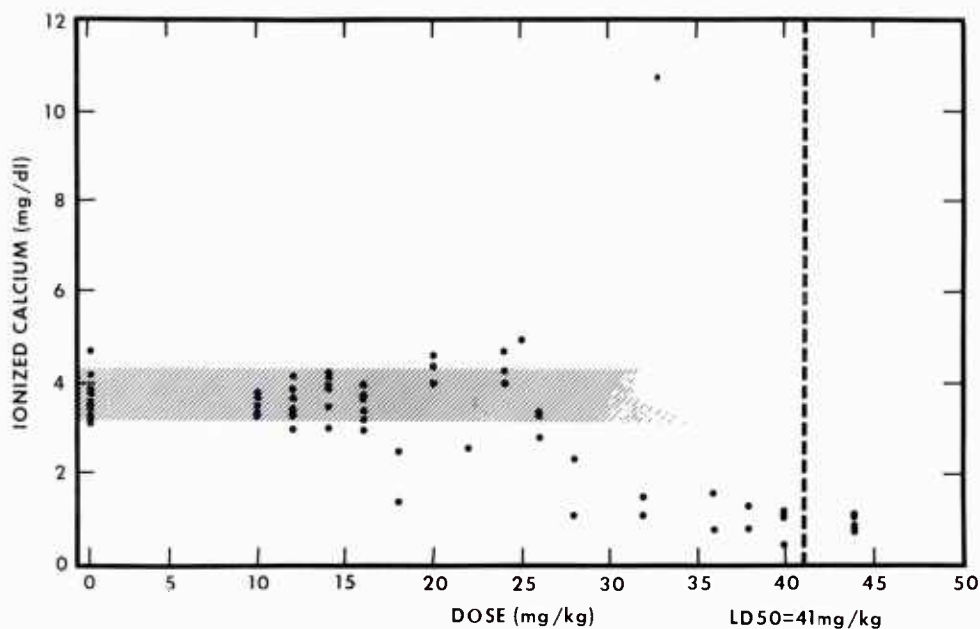
DOSE (mg/kg)

LD50=41mg/kg

Figure  6.  Serum ionized calcium levels 5 minutes following intravenous injection of pyrophosphate.  Each point represents the mean of 2 to 10 animals.  Hatched area represents normal range ($\overline{x}$ ± 2 S.D.) of control rats.

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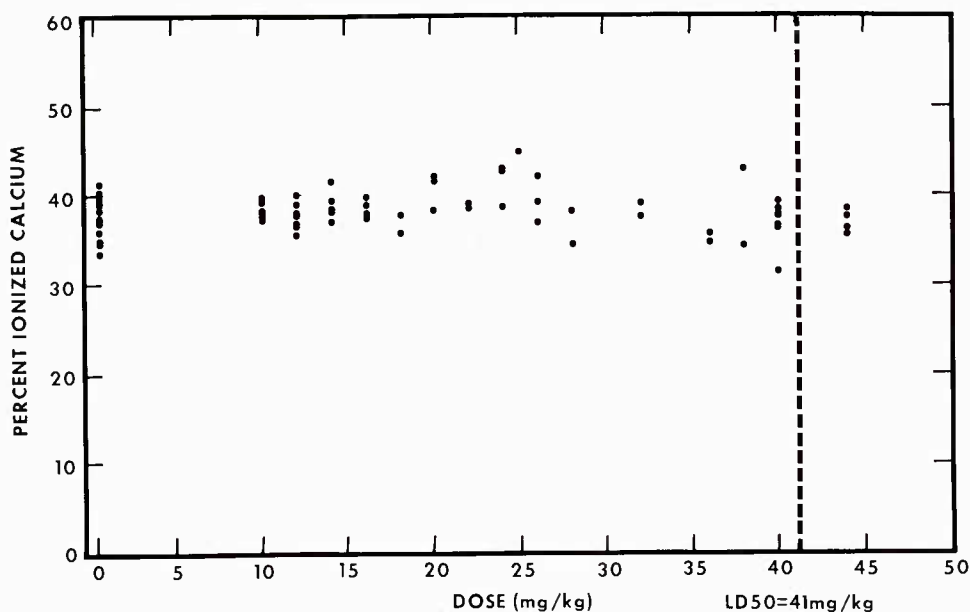
Figure 7.  Percent ionized calcium values.  Each point represents
the mean of 2 to 10 animals.

arrhythmia invariably hindered the analysis, and always occurred at a time when the

Q-T interval occupied approximately 60 percent of the cardiac cycle, as has previously

been observed.[7]  Cardiac arrest occurred shortly thereafter.

Controls.  Comparisons of serum calcium and protein levels were made between

10 control (without anesthesia) and 10 anesthetized Sprague-Dawley rats, weighing

400 to 450 grams.  The blood was drawn 15 minutes after the anesthetized animals

had received 25 mg of sodium pentobarbital intraperitoneally.  The serum calcium

and protein levels were obtained using the same techniques as previously described.

The results are shown in Table II.  The only significant difference observed between

anesthetized and unanesthetized animals was the significantly higher level of total pro-

tein in the unanesthetized animals, as has previously been observed.[9]  Use of saline

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Table II. Average Serum Calcium and Protein Levels
in Control and Anesthetized Rats

| | Controls without anesthesia[*] ± SD | With anesthesia[*] ± SD | Significance |
|---|---|---|---|
| Total calcium (mg/dl) | 9.19 ± 1.36 | 9.52 ± .34 | NS[+] |
| Bound calcium (mg/dl) | 5.53 ± .85 | 5.97 ± .29 | NS |
| Ionized calcium (mg/dl) | 3.65 ± .52 | 3.55 ± .23 | NS |
| Protein (g/dl) | 9.53 ± .35 | 8.31 ± .37 | (P < .001) |

* Number = 10
+ NS = not significant

in conjunction with anesthesia produced no additional effect upon the calcium or protein values.

Since the formula for calculating the ionized calcium levels uses the total protein values, the reported absolute values reflect the effect of anesthesia noted in this study.

## IV. DISCUSSION

The acute clinical manifestations encountered when a toxic dose of technetium-99m Sn-pyrophosphate is administered as a single intravenous bolus appear to be centered around two severe biochemical disturbances. They are (1) reduction of the plasma concentration of both ionized and total calcium and (2) a metabolic acidosis. The changes in the electrocardiogram and chemical analysis consistent with hypocalcemia were found to exist at a dose level, based on body weight, lower than that for the acute $LD_{50}$. It seems, therefore, that the use of the $LD_{50}$ as the primary end-point toxicologic analysis of the pyrophosphate compounds is not as meaningful as the evaluation

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of the associated hypocalcemia. Systemic metabolic acidosis developed sharply at a dosage similar to that found for the acute $LD_{50}$. Gosselin et al. have drawn attention to the fact that the method of the development of the systemic metabolic acidosis by polymeric phosphates is secondary to the enzymatic hydrolysis of these compounds in vivo.[7] This enzymatic hydrolysis releases unneutralized orthophosphoric acid residues. Except for pyrophosphate,[7] metabolic acidosis is the most important toxic response from phosphate agents administered intraperitoneally. However, hypocalcemia is the main toxic effect of technetium-99m Sn-pyrophosphate when administered as a single intravenous bolus. For doses in excess of the $LD_{50}$ level, however, the $PCO_2$ level shows a significant drop signifying that at these doses metabolic acidosis plays more of a primary role in the toxicology of pyrophosphate.

Toxicity of tin and tin colloids has previously been evaluated by Fischer.[6] He has demonstrated that as much as 350 mg/kg of body weight of tin do not produce any histopathological damage in rabbits. The relatively minute amount of tin used in the preparation of $^{99m}Tc$ Sn-pyrophosphate is so small that no toxic effects from the tin content of this agent should be encountered. Administration of the saline vehicle in quantities used for each dosage tested produced no abnormal effects.

It was interesting to note that polyphosphate was 1.4 times as toxic as pyrophosphate on an $LD_{50}$ basis but again evaluation of the hypocalcemic and acidosis potential of $^{99m}Tc$ Sn-polyphosphate must be made for a true comparison. If both the polyphosphate and pyrophosphate agents are serially diluted in normal saline and administered slowly or by intravenous drip infusion or conjointly with $CaCl_2$, the $LD_{50}$ level and clinical manifestations as previously described could be raised. It was found, however,

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that slow administration or dilution of the pyrophosphate agent in normal saline yields poor bone images and, therefore, it is not felt that the method of administration should be changed. A pilot $LD_{50}$ study using pyrophosphate and polyphosphate in 30 dogs, 12 pigs, 15 rabbits, and 200 Sprague-Dawley rats revealed an $LD_{50}$ range of 40 mg/kg to 70 mg/kg upon rapid intravenous injections, and from 70 mg/kg to 100 mg/kg using a slower injection rate of 2 ml per minute.[3] It appears, therefore, that the method of administration of these phosphate compounds plays a significant role in their toxic capabilities.

## V. SUMMARY

Two severe biochemical disturbances appear to explain the acute clinical manifestations of toxic doses of $^{99m}$Tc pyrophosphate when administered as a single intravenous bolus. These are (1) reduction in the serum concentration of both total and ionized calcium and (2) metabolic acidosis. The electrocardiogram and chemical analysis revealed that the hypocalcemic effect occurs at a much lower dosage than for the $LD_{50}$. Polyphosphate was found to be more toxic by the $LD_{50}$ analysis than pyrophosphate. It is emphasized that the hypocalcemic toxic effects should be the determining factor in deciding what dose of $^{99m}$Tc Sn-pyrophosphate should be administered for bone scanning. At the dose level we employ (.32 mg/kg) we have a safety factor of 40 to 1 with regard to the first signs of hypocalcemia by electrocardiographic measurements (i.e., 2 mg/kg). On this basis we recommend an injection of no more than 1 mg/kg to maintain a safety factor of around 10 to 1.

Systemic metabolic acidosis plays a less significant role in the toxic manifestations of this agent at the dose levels usually administered to patients (i.e., around

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0.3 mg/kg). We therefore feel that this agent is a safe radiopharmaceutical for bone scanning but emphasize that knowledge of the causes of its toxic clinical manifestations, and the safe dose levels which can be administered are necessary before one uses $^{99m}$Tc Sn-pyrophosphate as a bone scanning radiopharmaceutical.

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13. ABSTRACT

Following the administration of toxic doses of technetium-99m Sn-pyrophosphate in rats, clinical manifestations are consistent with tetany and metabolic acidosis. To evaluate these phenomena, the acute $LD_{50}/5$ minutes was determined and serum calcium and arterial plasma $PCO_2$ were recorded and compared to the acute $LD_{50}/5$ minutes. The $LD_{50}/5$ minutes for polyphosphate was found to be $29.4 \pm 2.0$ mg/kg body weight. The $LD_{50}/5$ minutes for pyrophosphate was found to be $41.0 \pm 1.6$ mg/kg body weight (2 S.D.). There was no significant difference between the slopes of the two compounds. When acute $LD_{50}$'s were compared, polyphosphate was 1.4 times as toxic as pyrophosphate. Arterial blood $PCO_2$ remained between 30 and 40 torr until dosages of pyrophosphate exceeded 35 mg/kg body weight, after which it fell sharply. Serum calcium was monitored by atomic absorption spectroscopy and electrocardiography. There was a significant drop in the ionized and bound calcium beginning at concentrations of an administered dose of 20 mg/kg body weight. However, tetany was not apparent until 22 mg/kg body weight of pyrophosphate were given. Therefore, evaluation of the toxic effects of phosphate agents should not be limited to determination of $LD_{50}$ alone, but should include appropriate biochemical measurements in blood.

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